

WHAT IS CLAIMED IS:

1. A recombinant DNA vector containing a nucleotide sequence that encodes a Flk-1 operatively associated with a regulatory sequence that controls gene expression in a host.

2. A recombinant DNA vector containing a nucleotide sequence that encodes a Flk-1 fusion protein operatively associated with a regulatory sequence that controls gene expression in a host.

3. An engineered host cell that contains the recombinant DNA vector of Claims 1 or 2.

4. An engineered cell line that contains the recombinant DNA expression vector of Claim 1 and expresses Flk-1.

5. The engineered cell line of Claim 3 which expresses the Flk-1 on the surface of the cell.

6. An engineered cell line that contains the recombinant DNA expression vector of Claim 2 and expresses the Flk-1 fusion protein.

7. The engineered cell line of Claim 6 that expresses the Flk-1 fusion protein on the surface of the cell.

8. A method for producing recombinant Flk-1, comprising:

(a) culturing a host cell transformed with the recombinant DNA expression vector of Claim 1 and which expresses the Flk-1; and

- (b) recovering the Flk-1 gene product from the cell culture.

5 9. A method for producing recombinant Flk-1 fusion protein, comprising:

- (a) culturing a host cell transformed with the recombinant DNA expression vector of Claim 2 and which expresses the Flk-1 fusion protein; and
- 10 (b) recovering the Flk-1 fusion protein from the cell culture.

15 10. An isolated recombinant Flk-1 receptor protein.

11. A fusion protein comprising Flk-1 linked to a heterologous protein or peptide sequence.

20 12. An oligonucleotide which encodes an antisense sequence complementary to a portion of the Flk-1 nucleotide sequence, and which inhibits translation of the Flk-1 gene in a cell.

25 13. The oligonucleotide of Claim 12 which is complementary to a nucleotide sequence encoding the amino terminal region of the Flk-1.

30 14. A monoclonal antibody which immunospecifically binds to an epitope of the Flk-1.

15. The monoclonal antibody of Claim 14 which competitively inhibits the binding of VEGF to the Flk-1.

35

16. The monoclonal antibody of Claim 14 which is linked to a cytotoxic agent.

17. The monoclonal antibody of Claim 14 which is  
5 linked to a radioisotope.

18. A method for screening and identifying antagonists of VEGF, comprising:

- 10 (a) contacting a cell line that expresses Flk-1 with a test compound in the presence of VEGF; and
- (b) determining whether the test compound inhibits the binding and cellular effects of VEGF on the cell line,
- 15 in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of VEGF on the cell line.

19. A method for screening and identifying  
20 agonists of VEGF, comprising:

- (a) contacting a cell line that expresses the Flk-1 with a test compound in the presence and in the absence of VEGF;
- 25 (b) determining whether, in the presence of VEGF, the test compound inhibits the binding of VEGF to the cell line; and
- (c) determining whether, in the absence of the VEGF, the test compound mimics the cellular effects of VEGF on the cell
- 30 line,
- in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of VEGF on the cell line.

35

20. The method according to Claims 18 or 19 in which the cell line is a genetically engineered cell line.

5        21. The method according to Claims 18 or 19 in which the cell line endogenously expresses the Flk-1.

22. A method for screening and identifying antagonists of VEGF comprising:

- 10            (a) contacting Flk-1 protein with a random peptide library such that Flk-1 will recognize and bind to one or more peptide species within the library;
- 15            (b) isolating the Flk-1/peptide combination;
- (c) determining the sequence of the peptide isolated in step c; and
- 20            (d) determining whether the test compound inhibits the binding and cellular effects of VEGF,

in which antagonists are identified as those peptides that inhibit both the binding and cellular effects of VEGF.

25        23. A method for screening and identifying agonists of VEGF comprising:

- (a) contacting Flk-1 protein with a random peptide library such that Flk-1 will recognize and bind to one or more peptide species within the library;
- 30            (b) isolating the Flk-1/peptide combination;
- (c) determining the sequence of the peptide isolated in step c; and

35

(d) determining whether, in the absence of the VRGF, the peptide mimics the cellular effects of VEGF, in which agonists are identified as those peptides  
5 that inhibit the binding but mimic the cellular effects of Flk-1.

24. The method according to Claims 22 or 23 in which the Flk-1 protein is genetically engineered.  
10

25. A method of modulating the endogenous enzymatic activity of the tyrosine kinase Flk-1 receptor in a mammal comprising administering to the mammal an effective amount of a ligand to the Flk-1  
15 receptor protein to modulate the enzymatic activity.

26. The method of Claim 25 in which the ligand to the Flk-1 receptor is VEGF.

20 27. The method of Claim 25 in which the ligand to the Flk-1 receptor is a VEGF agonist.

28. The method of Claim 25 in which the ligand to the Flk-1 receptor is an antagonist of VEGF.  
25

29. The antagonist of Claim 28 that is a monoclonal antibody which immunospecifically binds to an epitope of Flk-1.

30 30. The antagonist of Claim 28 that is a soluble Flk-1 receptor.

31. The method of Claim 25 in which the enzymatic activity of the receptor protein is  
35 increased.

32. The method of Claim 25 in which the enzymatic activity of the receptor protein is decreased.

5        33. The method of Claim 31 in which the ligand stimulates endothelial cell proliferation.

34. The method of Claim 32 in which the ligand inhibits endothelial cell proliferation.

10

35. The method of Claim 32 in which the ligand inhibits angiogenesis.

36. A recombinant vector containing a nucleotide  
15 sequence that encodes a truncated Flk-1 which has dominant-negative activity which inhibits the cellular effects of VEGF binding.

37. The recombinant vector of claim 36  
20 containing a nucleotide sequence encoding amino acids 1 through 806 of Flk-1.

38. The recombinant vector of claim 36 in which the vector is a retrovirus vector.

25

39. The recombinant vector of claim 38 containing a nucleotide sequence encoding amino acids 1 through 806 of Flk-1.

40. An engineered cell line that contains the recombinant DNA vector of Claim 36 and expresses truncated Flk-1.

41. An engineered cell line that contains the  
35 recombinant vector of Claim 38 or 39 and produces

infectious retrovirus particles expressing truncated Flk-1.

42. An isolated recombinant truncated Flk-1  
5 receptor protein which has dominant-negative activity  
which inhibits the cellular effects of VEGF binding.

43. A method of modulating the cellular effects  
of VEGF in a mammal comprising administering to the  
10 mammal an effective amount of truncated Flk-1 receptor  
protein which inhibits the cellular effects of VEGF  
binding.

44. A method of identifying a compound that  
15 inhibits phosphorylation of Flk-1 receptor comprising:  
(a) measuring Flk-1 receptor autophosphorylation  
in the presence of VEGF and the compound;  
(b) measuring Flk-1 receptor autophosphorylation  
in the presence of VEGF in the absence of the  
20 compound; and  
(c) determining the difference in Flk-1  
autophosphorylation in the presence and absence of the  
compound, wherein decreased autophosphorylation  
indicates inhibition.

25 45. A compound that inhibits autophosphorylation  
of Flk-1 receptor.

46. The compound according to claim 45 that is  
30 compound A14.